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Validity of Fecal Coliforms, Total Coliforms, and Fecal Streptococci as Indicators of Viruses in Chlorinated Primary Sewage Effluents

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Quantities of combined chlorine that usually destroyed more than 99.999% of the indigenous fecal coliforms, total coliforms, and fecal streptococci in primary sewage effluents destroyed only 85 to 99% of the indigenous viruses present. Viruses were recovered from five of eight chlorinated primary effluents from which fecal coliforms were not recovered by standard most-probable-number procedures. The limited volumes of such chlorinated effluents that can be tested for indicator bacteria with currently available multiple-tube and membrane filter technics restrict the value of fecal coliforms, fecal streptococci, and even total coliforms as indicators of viruses in these effluents. Although fecal coliforms and fecal streptococci are useful indicators of viruses in effluents from which these bacteria are recovered, the absence of these bacteria and even total coliforms from disinfected effluents (in standard tests) does not assure that viruses are also absent.

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Validity of Fecal Coliforms, Total Coliforms, and Fecal Streptococci as Indicators of Viruses in Chlorinated Primary Sewage Effluents

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Quantities of combined chlorine that usually destroyed more than 99.999% of the indigenous fecal coliforms, total coliforms, and fecal streptococci in primary sewage effluents destroyed only 85 to 99% of the indigenous viruses present. Viruses were recovered from five of eight chlorinated primary effluents from which fecal coliforms were not recovered by standard most-probable-number procedures. The limited volumes of such chlorinated effluents that can be tested for indicator bacteria with currently available multiple-tube and membrane filter techniques restrict the value of fecal coliforms, fecal streptococci, and even total coliforms as indicators of viruses in these effluents. Although fecal coliforms and fecal streptococci are useful indicators of viruses in effluents from which these bacteria are recovered, the absence of these bacteria and even total coliforms from disinfected effluents (in standard tests) does not assure that viruses are also absent.

Fecal coliforms are the standard bacterial indicator of fecal pollution in wastewater and other waters. Fecal streptococci serve as indicators of such pollution in some situations also. Total coliforms, which include the fecal coliforms, are the standard indicator of pollution in drinking water.

That fecal coliforms, fecal streptococci, and even total coliforms are not always adequate indicators of viruses in chlorinated primary sewage effluents and probably in other effluents also is demonstrated herein.

MATERIALS AND METHODS

Pretreatment of sewage effluents. Fresh primary sewage effluents were filtered through 293-mm-diameter membrane (type AP20, Millipore Corp.) prefilters seated on 0.45-μm (average pore size) membranes (type HA, Millipore Corp.), both of which had been treated with 1 liter of 0.1% Tween 80 and then washed with 2 liters of sterile distilled water. Primary effluents rather than secondary effluents were used in these studies to avoid some of the quantification problems inherent in the concentration of large volumes of secondary effluents.

Recovery of viruses. $MgCl_2$ (final concentration, 0.0055 M) was added to the AP20-filtered effluent, the pH of the sample was lowered to 3.5 with HCl, and the sample was filtered through a 142-mm, 0.45-μm membrane (Millipore HA). Viruses that adsorbed to the membrane were eluted with 40 ml of 3% beef extract in distilled water (2, 3, 10, 14). The eluate was inoculated onto cultures of BGM cells (5).

Cell cultures. BGM cells, a continuous line derived from an African green monkey (*Cercopithecus aethiops*) kidney, were cultivated as described earlier (5). The methods for the preparation of cell monolayer cultures and for the plaque overlay technique have also been described (5). Plaques that formed on inoculated monolayers were counted at intervals of several days for as long as the integrity of the cell sheet permitted, usually 2 to 3 weeks. Counts were expressed as plaque-forming units.

Determination of total coliforms, fecal coliforms, and fecal streptococci. Bacterial tests were performed in accordance with the procedures described in *Standard Methods for the Examination of Water and Wastewater* (6).

Total coliforms. Except where otherwise indicated, total coliform numbers were determined by the multiple-tube, most-probable-number (MPN) technique. Five tubes were used for each dilution. Lauryl tryptose broth was used for the presumptive test. Cultures in which gas formed within 48 h after inoculation were confirmed in brilliant green lactose bile broth.

Fecal coliforms. Except where otherwise indicated, fecal coliform numbers were determined by the multiple-tube MPN technique also. Positive presumptive total coliform cultures were confirmed in EC medium at 44.5°C.

Fecal streptococci. Fecal streptococcus numbers were determined by membrane filter procedures on autoclaved KF medium. Counts were expressed as colony-forming units.

Chlorination procedure. Primary sewage ef-

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fluents were chlorinated with predetermined quantities of reagent-grade sodium hypochlorite (NaOCl). The NaOCl was mixed vigorously with each effluent for 15 min at room temperatures (20 to 24°C). Each liter of chlorinated effluent was neutralized with 0.8 ml of 10% sodium thiosulfate.

Chlorine determination. Residual chlorine in each chlorinated primary effluent was determined amperometrically on 200-ml samples by the back-titration procedure with phenylarseneoxide at pH 3.5 to 4.2 in the presence of KI (6).

RESULTS

Primary effluents from several sewage treatment plants were chlorinated to final combined residuals of 11.1 to 23.3 mg/liter at pH levels of 8.2 to 9.2 (Table 1). From levels of 109 to 427 plaque-forming units/liter, virus titers in the effluents were reduced in numbers by 85 to 99% in 15 min (Table 2). From levels of 1.7×10^4 to 1.3×10^7 MPN/100 ml, fecal coliforms were reduced in numbers by 99.95 to >99.99998% in the same time (Table 3). The numbers of total coliforms (Table 4) and fecal streptococci (Table 5) were reduced extensively under the same conditions.

Although the numbers of viruses in the primary effluents never exceeded 427 plaque-forming units/liter, some viruses were always detected in all of the eight effluents tested after 15 min of chlorination (Table 2). In five of the eight tests, however, no fecal coliforms were recovered from the chlorinated effluents by standard multiple-tube MPN procedures (Table 3). The numbers of total coliforms in the primary effluents averaged nearly an order of magnitude greater than the numbers of fecal coliforms, and although some total coliforms were always recovered from these effluents after 15 min of chlorination, it was clear from several of the tests that small increases in the chlorination-time-of-contact couplings would have reduced the numbers of total coliforms to undetectable levels (Table 4). Fecal streptococci, their numbers averaging perhaps an order of magnitude lower than the numbers of fecal coliforms in the primary effluent, were not recovered from six of the seven effluents after 15 min of chlorination (Table 5). (Although it now appears that two to five times as many fecal streptococci might have been recovered had the KF medium been sterilized by boiling rather than by autoclaving at 121°C for 10 min, as it was, even five times the numbers of fecal streptococci recovered would change little.)

The ratios of fecal coliforms to viruses ranged from 8.9×10^1:1 to 7.2×10^4:1 in the primary effluents before chlorination and from <0.06:1 to 3.4:1 in those same effluents after 15 min of chlorination. In seven of the eight effluents, fecal coliform-to-virus ratios exceeded 10^4:1 before chlorination but were less than 1:1 in seven of eight effluents after 15 min of chlorination (Table 6). Total coliform-to-virus ratios in the primary effluents ranged from 1.5×10^4:1 to $\geq 5.7 \times 10^5$:1 before chlorination and from 0.35:1 to

TABLE 2. *Destruction of viruses in primary sewage effluents by combined chlorine in 15 min at 20 to 24°C*

Date	Combined chlorine[a] (mg/liter)	Viruses (PFU[b]/liter)		% Destruction
		Primary effluent	Chlorinated primary effluent	
14 Aug.	11.8–11.1	192	22	89
27 Aug.	16.0–14.0	427	36	92
4 Sept.	16.2–14.9	178	8	96
10 Sept.	16.0–17.5	422	20	95
25 Sept.	18.0–18.0	109	16	85
1 Oct.	23.3–23.3	222	6	97
30 Oct.	18.6–20.7	210	2	99
6 Nov.	21.2–21.2	185	3.5	98

[a] Initial–15-min residual (see Table 1).
[b] PFU, Plaque-forming units.

TABLE 1. *Characteristics of primary sewage plant effluents that affected destruction of bacteria and viruses by combined chlorine*

Date	Sewage treatment plant	pH		Combined chlorine (mg/liter)		NH_3-N (mg/liter)	Total organic carbon (mg/liter)
		Primary effluent	Chlorinated primary effluent	Initial	After 15 min		
14 Aug.	Sycamore	7.7	8.3	11.8	11.1	5.8	13.4
27 Aug.	Sycamore	7.5		16.0	14.0	10.9	28.5
4 Sept.	Sycamore	7.3	8.5	16.2	14.9	4.6	29.0
10 Sept.	Sycamore	7.2	8.2	16.0	17.5	15.7	34.0
25 Sept.	Sycamore	7.6	8.5	18.0	18.0	15.1	62.0
1 Oct.	Sycamore	7.7	8.6	23.3	23.3	13.8	39.0
30 Oct.	Mill Creek	7.2	8.7	18.6	20.7	19.0	71.0
6 Nov.	Muddy Creek	7.8	9.2	21.2	21.2	12.8	29.0

TABLE 3. *Destruction of fecal coliforms in primary sewage effluents by combined chlorine in 15 min at 20 to 24°C*

Date	Combined chlorine[a] (mg/liter)	Fecal coliforms (MPN/100 ml)		% Destruction
		Primary effluent	Chlorinated primary effluent	
14 Aug.	11.8–11.1	1.7×10^4	9	99.95
27 Aug.	16.0–14.0	7.0×10^6	<2	>99.99997
4 Sept.	16.2–14.9	2.4×10^{6}[b]	27	99.999
10 Sept.	16.0–17.5	4.9×10^6	<2	>99.99996
25 Sept.	18.0–18.0	7.9×10^6	<2	>99.99997
1 Oct.	23.3–23.3	2.8×10^6	2	99.99993
30 Oct.	18.6–20.7	1.3×10^7	<2	>99.99998
6 Nov.	21.2–21.2	2.2×10^6	<2	>99.99991

[a] Initial–15-min residual (see Table 1).
[b] Fecal coliform count given as colony-forming units per 100 ml.

TABLE 4. *Destruction of total coliforms in primary sewage effluents by combined chlorine in 15 min at 20 to 24°C*

Date	Combined chlorine[a] (mg/liter)	Total coliforms (MPN/100 ml)		% Destruction
		Primary effluent	Chlorinated primary effluent	
14 Aug.	11.8–11.1	2.8×10^6	23	99.9992
27 Aug.	16.0–14.0	3.3×10^7	23	99.99993
4 Sept.	16.2–14.9	1.1×10^{7}[b]	130	99.999
10 Sept.	16.0–17.5	$\geq 2.4 \times 10^8$	7	≥99.999997
25 Sept.	18.0–18.0	2.4×10^7	22	99.99991
1 Oct.	23.3–23.3	1.3×10^7	79	99.9994
30 Oct.	18.6–20.7	4.9×10^7	11	99.99998
6 Nov.	21.2–21.2	1.5×10^7	5	99.99997

[a] Initial–15-min residual (see Table 1).
[b] Total coliform count given as colony-forming units per 100 ml.

16:1 after 15 min of chlorination. In three of the chlorinated effluents, the total coliform-to-virus ratios were equal to or less than 1:1, and in two such effluents the ratio was only 1.4:1. In the seven primary effluents tested, fecal streptococcus-to-virus ratios ranged from 5.2×10^2:1 to 8.1×10^3:1 before chlorination and were always less than 1.1:1 after 15 min of chlorination.

DISCUSSION

Free chlorine (HOCl and OCl^-) in the chlorinated primary effluents disappeared so quickly that it could not be measured. The ammonia chloramine that forms at pH levels of 8.2 to 9.2 in effluents with low chlorine-to-ammonia ratios is primarily monochloramine, but with the large amounts of organic carbon in these effluents, a lot of it undoubtedly nitrogenous, much of the chloramine was probably organic. Monochloramine destroys bacteria and viruses much more

TABLE 5. *Destruction of fecal streptococci in primary sewage effluents by combined chlorine in 15 min at 20 to 24°C*

Date	Combined chlorine[a] (mg/liter)	Fecal streptococci (CFU[b]/100 ml)		% Destruction
		Primary effluent	Chlorinated primary effluent	
14 Aug.	11.8–11.1	3.1×10^5	10	99.997
27 Aug.	16.0–14.0	2.2×10^5	<4	>99.998
10 Sept.	16.0–17.5	5.4×10^5	<4	>99.9993
25 Sept.	18.0–18.0	7.2×10^5	<4	>99.9994
1 Oct.	23.3–23.3	1.8×10^6	<4	>99.9998
30 Oct.	18.6–20.7	2.4×10^5	<2	>99.9992
6 Nov.	21.2–21.2	6×10^5	<4	>99.9993

[a] Initial–15-min residual (see Table 1).
[b] CFU, Colony-forming units.

TABLE 6. *Ratios of fecal coliforms, total coliforms, and fecal streptococci to viruses in primary sewage effluents before and after chlorination for 15 min at 20 to 24°C*[a]

Date	Combined chlorine[b] (mg/liter)	Viruses (PFU/liter)		Fecal coliforms-viruses (MPN/100 ml:PFU/liter)		Total coliforms-viruses (MPN/100 ml:PFU/liter)		Fecal streptococci-viruses (CFU/100 ml:PFU/liter)	
		Primary effluent	Chlorinated primary effluent	Primary effluent	Chlorinated primary effluent	Primary effluent	Chlorinated primary effluent	Primary effluent	Chlorinated primary effluent
14 Aug.	11.8–11.1	192	22	8.9×10^1:1	0.41:1	1.5×10^4:1	1.0:1	1.6×10^3:1	0.45:1
27 Aug.	16.0–14.0	427	36	1.6×10^4:1	<0.06:1	7.7×10^6:1	0.64:1	5.2×10^2:1	<0.11:1
4 Sept.	16.2–14.9	178	8	1.3×10^4:1[c]	3.4:1	6.2×10^6:1[c]	16:1		
10 Sept.	16.0–17.5	422	20	1.2×10^4:1	<0.10:1	$\geq 5.7 \times 10^6$:1	0.35:1	1.3×10^3:1	<0.02:1
25 Sept.	18.0–18.0	109	16	7.2×10^4:1	<0.12:1	2.2×10^5:1	1.4:1	6.6×10^3:1	<0.25:1
1 Oct.	23.3–23.3	222	6	1.3×10^4:1	0.33:1	5.9×10^4:1	13.1	8.1×10^3:1	<0.67:1
30 Oct.	18.6–20.7	210	2	6.2×10^4:1	<1.0:1	2.5×10^5:1	5.5:1	1.1×10^3:1	<1.0:1
6 Nov.	21.2–21.2	185	3.5	1.2×10^4:1	<0.57:1	8.1×10^4:1	1.4:1	3.2×10^3:1	<1.1:1

[a] Based on data in Tables 2–5. PFU, Plaque-forming units; CFU, colony-forming units.
[b] Initial–15-min residual (see Table 1).
[c] CFU/100 ml:PFU/liter.

slowly than HOCl and OCl^- do (11), and organic chloramines, generally, work even more slowly. Given sufficient concentration and time, many of the chloramines can destroy all of the vegetative bacteria and viruses in an effluent. However, the differences in the rates at which bacteria and viruses are destroyed by chloramines are enough that with certain concentration–time couplings, most and perhaps all of the indicator bacteria can be destroyed with many viruses still surviving. In the experiments reported herein, chlorine levels that destroyed all or nearly all of the fecal coliforms and fecal streptococci in primary effluents were repeatedly achieved. Viruses survived in all of these effluents even though fecal coliform counts were well within the limits permitted for recreational waters (200 to 400 MPN/100 ml; 13) and for approved shellfish-growing waters (14 MPN/100 ml; 7, 13). Even if total coliform counts, which have no sanitary significance (1), had been used as the criterion, the counts would have been well within those generally accepted for fecal coliforms in approved recreational and shellfish-growing waters.

In all of these tests, the ratios of bacteria to viruses are based on volumes of 100 ml for the bacteria and 1 liter for the viruses. The methods and reporting for the bacteria are standard (6). There are as yet no standard methods for recovering viruses from sewage effluents. The quantities of effluents used for such recoveries range from a few milliliters in geographic areas usually hot and with poor sanitation, where sewage contains large numbers of viruses, to 4 liters or more in areas where sewage is less concentrated, the populations are more sanitary, and the numbers of viruses in sewage are somewhat smaller. The quantities of viruses in sewage are generally reported now on a per-liter basis. Since the smallest number of viruses that can be detected may be sufficient to produce infection in susceptible individuals who ingest them (8, 9), the presence of viruses in liter or much greater quantities of water that may become a part of recreational or shellfish-growing waters represents a possible hazard to the consumer and is significant. Unfortunately, it is not now possible to sample even liter quantities of effluent quality waters for indicator bacteria. The multiple-tube MPN procedure must be used for fecal and total coliforms in chlorinated effluents, because injured coliforms cannot be recovered as readily under the stressful conditions of the membrane filtration test as under the enriched, less toxic conditions of the MPN test. A maximum of 10 ml of test effluent is usually used in the MPN procedures, and this was the maximum volume used in the MPN tests reported herein. Even in membrane filter procedures, such as the KF procedure used for fecal streptococci, colloids and other suspended matter often prevent filtration of more than 25 to 50 ml of effluent. Although it would clearly be useful to sample much larger quantities of chlorinated effluents for indicator bacteria, current technology is not permissive of this and thereby allows that effluents (and other waters as well) which contain viruses are adjudged safe.

When it is present, the fecal coliform remains a reasonable indicator for viruses in chlorinated primary effluents (and probably in all other effluents). However, the numbers of fecal coliforms currently promulgated as safe levels for effluents (200 to 400 MPN/100 ml; 12) and for recreational (200 to 400 MPN/100 ml; 13) and shellfish-growing (14 MPN/100 ml; 7, 13) waters may not constitute safe levels (4). In chlorinated effluents, fecal coliforms (and other indicator bacteria) are destroyed so much more quickly than viruses that it is possible for all or most of the indicator bacteria to be destroyed long before all of the viruses are destroyed. This is especially true when the volume of effluent examined for indicator bacteria is limited both by the volume impositions of the standard methods and to current practicality, and the volume examined for viruses is not limited by such constraints.

Thus, chlorinated effluents (and undoubtedly otherwise disinfected effluents) that do not contain fecal coliforms (or probably any other nonsporeforming indicator bacteria) by standard tests may contain viruses and may thereby be unsafe.

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